

Synthesis of 7-Azafolic Acid (1)

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Received July 23, 1973

Sir:

In an earlier investigation of folic acid analogs, modifications in the pyrimidine ring resulted in the preparation of 3-deazamethotrexate (2). This compound was active against leukemia L1210 in mice presumably because of the inhibition of folic reductase (FR) (3). Recently work has been directed toward the preparation of analogs of folic acid that inhibit, not FR, but other enzymatic steps in which folates serve as cofactors (4). In the search for such inhibitors the pyrazine ring of folic acid was modified to give 7-azafolic acid 6 (5). This compound contains the 2-amino-4-oxypyrimido moiety of folic acid and might serve as a substrate for FR, producing 7-azatetrahydrofolic acid, a potential inhibitor of the one carbon transfers involving tetrahydrofolates. Mild chemical reducing agents (sodium hydrosulfide) are known to reduce the triazine ring of 7-azapteridines (6) but not the pyrazine ring of pteridines (7) suggesting that the enzymatic reduction of 7-azafolic acid to 7-azadihydro- and 7-azatetrahydrofolic acids might occur as readily as

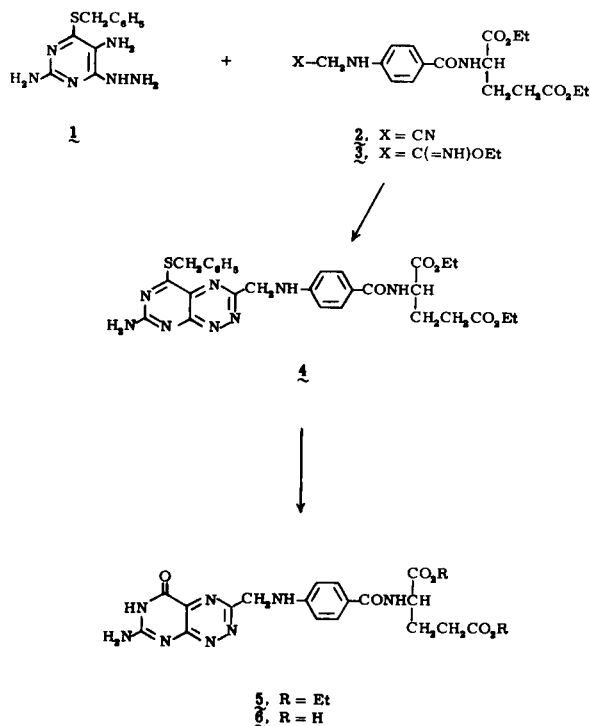
the reduction of folic acid to dihydro- and tetrahydrofolic acids.

Alkylation of diethyl *p*-aminobenzoyl-L-glutamate (8) with cyanomethyl *p*-toluenesulfonate (9) in refluxing dioxane for 120 hours gave the cyanomethyl derivative 2 (m.p., 95°), which was purified by column chromatography (silica gel H) and recrystallization from a mixture of benzene and hexane. Addition of ethanol to the cyano group of 2 in the presence of sodium ethoxide gave the imino ether 3, (10) which was condensed *in situ* at room temperature with the 5-amino-4-hydrazinopyrimidine 1 (6b) to give directly an 81% crude yield of the 7-azapteridine 4. Recrystallization of 4 from ethanol gave the analytically pure sample (m.p., 151°), the structure of which was confirmed by its uv, ir, and pmr spectra. Presumably, during the reaction a dihydro derivative of 4 is converted by air oxidation to the heteroaromatic system. A similar reaction of 1 with the imino ether obtained from ethyl *p*-[(cyanomethyl)amino]benzoate gave the corresponding 7-azapteridine (11).

Treatment of an aqueous DMSO solution of 4 with potassium bicarbonate at 90° for 18 hours followed by isolation of 5 and treatment of this product twice with oxygen-free 0.2 *N* sodium hydroxide at room temperature for 18 hours hydrolyzed the ester groups to give a 30.5% yield of 6 sesquihydrate, m.p. >264° with softening from ~240°.

As expected, 6 (I_{50} , 2×10^{-5} *M*) was a less effective inhibitor of pigeon liver FR than methotrexate (I_{50} , 1×10^{-8} *M*) (12). Disappointingly, the lack of activity when 6 was substituted for dihydrofolic acid in the test system indicated that 6 was not a substrate for FR. However, 4 at a concentration of 1×10^{-4} *M* produced a 9-fold increase in the rate of disappearance of NADPH as compared with that observed for the control. When this test was repeated in the absence of FR, a 2-fold increase was obtained. These results suggested that NADPH chemically reduced 4 in the absence of FR and that this reduction was accelerated in the presence of FR, presumably because 4 served as a substrate.

In contrast to the results described above, 6 (ED_{50} , 9.8×10^{-10} *M*) (13) was about as effective as methotrexate (ED_{50} , 6.6×10^{-10} *M*) (13) when tested against



Streptococcus faecium ATCC 8043 (14). Compound **4** (ED_{50} , $3.5 \times 10^{-6} M$) was less active in this test system. Although **6** might be a strong inhibitor of the FR of *S. faecium* and a weak inhibitor of this enzyme from pigeon liver because of species differences, it is possible that in the former another enzyme such as thymidylate synthetase is being inhibited. Currently the preparation of other 7-azapteridines for biologic evaluation is being investigated.

Acknowledgment.

The authors are indebted to Dr. R. F. Pittillo for the bacteriological testing and to Miss Suzanne Straight for the enzymological testing.

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